

FINAL TECHNICAL REPORT

GRANT #: N00014-02-1-0471

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INSTITUTION: The University of Arizona

GRANT TITLE: Glycopeptides as Analgesics: Non-Toxic Alternatives to Morphine for Combat Casualty Care

AWARD PERIOD: 1 May 2002 — 31 Dec 2005

OBJECTIVE: To develop a non-toxic alternative to morphine for use on the battlefield.

APPROACH: Glycosylation of delta-selective opioid agonists related to the endogenous peptide neurotransmitter Leu-enkephalin results in the incorporation of drug-like behavior. That is, the incorporation of sugars into peptide neurotransmitters leads to systemically available glycopeptides that can penetrate the blood-brain barrier (BBB) after iv, ip or im injection to produce potent and long-lasting analgesia. Furthermore, the balanced delta/mu-agonism displayed by these novel drugs resulted in a reduction of many narcotic side effects that are typical for opiates.

The initial studies involved the use of in vitro binding assays to opiate receptor subtypes, combined with in vivo studies in mice to simultaneously optimize performance of the drugs at the receptor, stability of the drug in vivo, and transport of the drug across the BBB.

ACCOMPLISHMENTS (throughout award period): We have produced 3 classes of glycopeptide drugs that have potential value in the clinic. The first class are the delta/mu-opioid agonist enkephalin analogues, illustrated by our lead compound Lactomorphin (MMP-2200 or ACR-150). In mice, these drugs showed minimal narcotic side effects, including abuse liability, constipation and sedation. The second class of glycopeptide drugs includes highly mu-selective opioid agonists, illustrated by LYM-100 or LYM-50. While these drugs still possess some narcotic side effects, including sedation, they are extremely potent and may have some advantages over fentanyl as a surgical anesthetic and for post-operative pain where sedation is required. The third class of opioid agonists includes the much larger endorphin-based glycopeptides that may eventually be useful for their non-analgesic effects. Since this falls outside the scope of our original aims, this work was discontinued until non-ONR support (NSF, NIH) can be obtained to pursue this line of inquiry.

Methods developed during the award period include improved synthetic methods for glycopeptides, and a new concept for the conversion of neurotransmitters (effective range of action = 10-100Å) into pharmaceuticals (effective range of action = mm-meters). This "biosition" concept is presently being explored and expanded in the context of a new NSF grant that allows us to explore and expand on this novel approach to drug design.

CONCLUSIONS: Glycopeptides analgesics are potent analgesics with limited abuse liability. Several of these are worthy lead compounds for the replacement of morphine in the field. Further studies on the pre-clinical and clinical development with ONR support are presently underway.

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SIGNIFICANCE: Our studies have provided a valuable new approach to the treatment of pain with minimal narcotic side effects. Furthermore, the use of glycosylation as a means of converting endogenous neurotransmitters into CNS drugs may represent a sea change in the design of drugs for the treatment of numerous disorders.

Two other companies, one CA based, and one based in Tucson, have proposed to work in collaboration with us to use glycosylation for other (non-analgesic) applications. At this point, this work is still in the formative stages.

PATENT INFORMATION: Several patents have been filed, and commercialization is in pursuit. Acretia Inc., located in the Boston metropolitan area, has acquired certain rights to our intellectual property and is helping us to further develop a drug that will be useful for traumatic pain on the battlefield, as well as the treatment of chronic pain associated with cancer.

1. PCT/04/0530 Filed 02/24/04 WO2004/075843

TITLE OF THE INVENTION: GLYCOSYLATED ENKEPHALIN AGENTS

2. PCT/TBD (UA Ref. #04/065) filed 03/29/05

TITLE OF THE INVENTION: GLYCOPEPTIDE ANALGESICS

3. DOCKET NO: 264413US

TITLE OF THE INVENTION: AMPHIPATHIC GLYCOPEPTIDES

AWARD INFORMATION: Several graduate student awards have been garnered: Michael Palian was awarded the *Marvel Award*, for overall research achievement in the Dept. of Chemistry at the University of Arizona. Mr. Charles M. Keyari was awarded the *O'Brien Award*, for research with membranes.

REFEREED PUBLICATIONS (for award period only, more are pending):

1. Palian, M.M. *et al.* (2003) Glycopeptide-Membrane Interactions: Glycosyl Enkephalin Analogs Adopt Turn Conformations by NMR and CD in Amphipathic Media. *J. Am. Chem. Soc.* 125: 5823-5831.

2. Elmagbari, N.O. *et al.* (2004) Antinociceptive Structure-Activity Studies with Enkephalin-Based Opioid Glycopeptides. *J. Pharm. Exp. Therap.* 311: 290-297.

3. Egleton, R.D. *et al.* (2005) Biousian glycopeptides penetrate the blood-brain barrier. *Tetrahedron: Asymm.* 16: 65-75.

4. Muthu, D. & Polt, R. (2005) New Prospects for Glycopeptide Based Analgesia: Glycoside-Induced Penetration of the Blood-Brain Barrier. *Current Drug Delivery* 2: 59-73.

5. Muthu, D. *et al.* (2005) Glycopeptides Related to β -Endorphin Adopt Helical Amphipathic Conformations in the Presence of Lipid Bilayers. *J. Am. Chem. Soc.* 127: 5435-5448.

6. Muthu, D. *et al.* (2005) Glycosylated Neuropeptides: A New Vista for Neuropsychopharmacology. *Med. Res. Rev.* 25, 557-585.

BOOK CHAPTERS, SUBMISSIONS, ABSTRACTS AND OTHER PUBLICATIONS (for total award period)

1. Eggleton RD, Hawkins BT, Bilsky EJ, *et al.* (2006) The role of blood brain barrier transport in the enhanced analgesia of glycopeptide opioids. *FASEB JOURNAL* 20 (4): A240-A241 Part 1 MAR 6.
2. Lowery J, Raymond T, Yeomans L, *et al.* (2006) In vivo pharmacology of MMP2200 a delta/mu opioid glycopeptide. *FASEB JOURNAL* 20 (4): A241-A241 Part 1 MAR 6.
3. Yeomans L, Muthu D, Keyari CM, *et al.* (2005) Mu-selective opioid glycopeptide that crosses the blood-brain barrier. *ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY* 229: U117-U117 063-MEDI Part 2 MAR 13.
4. Dhanasekaran M, Alves I, Keyari CM, *et al.* (2005) Drugs for the brain from the brain: Analgesia & behavioral effects of glycopeptides based on enkephalins & endorphins. *BIOPOLYMERS* 80 (4): 547-547.
5. Eggleton RD, Bilsky E, Polt RL (2005) Physiochemical analysis and CNS bioavailability of novel opioid amphipathic glycopeptides. *FASEB JOURNAL* 19 (4): A510-A510 Part 1 Suppl. S MAR 4.
6. Paolino RM, Lowery JJ, Yeomans L, *et al.* (2005) Comparison of Mu and delta/mu opioid glycopeptides in mouse models of anti nociception, locomotor activity, physical dependence and gastrointestinal transit. *FASEB JOURNAL* 19 (5): A1069-A1069 Part 2 Suppl. S MAR 7.
7. Polt R, Muthu D, Bilsky EJ, *et al.* (2004) Glycopeptide analgesics: Conformational and pharmacological characterization of O-linked glycosyl-enkephalins and glycosyl-endorphins. *ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY* 227: U150-U150 278-ORGN Part 2 MAR 28.
8. Polt R, Palian MM, Bilsky EJ, *et al.* (2003) Glycosylated enkephalins penetrate the blood-brain barrier adsorptive endocytosis - Analgesia superior to morphine. *ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY* 225: U322-U322 256-ORGN Part 2 MAR.

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13. ABSTRACT (Maximum 200 Words) Background: Endogenous opiate peptides (enkephalins and endorphins) are more potent and specific than morphine and congeners. Specificity is determined by the "address segment" while binding is determined by the "message segment." Incorporation of carbohydrates into the address segment results in improved biodistribution and enhanced penetration of the blood-brain barrier (BBB). Thus, glycosylation of mu- or delta-selective peptides allows the resulting glycopeptides to be used as potent and safer alternatives to classical opiates such as morphine. Morphine and other mu-selective agonists are immunosuppressants, while the glycopeptide enkephalins are selective delta-agonists, which are known to be immunostimulants. Hypothesis: Enkephalin transport and penetration of the blood-brain barrier can be determined by reversible binding to membranes, which may be manipulated by altering the amphipathicity of the address segment directly by introduction of hydrophilic sugar moieties, and lipophilic side chains. Conclusion: Glycopeptide analogues of enkephalins are viable drug candidates. Obstacles regarding the synthesis and design of analgesics have been overcome. Further studies are required in order to understand the clinical implications of their use both in a hospital setting, as well as on the battlefield, and also in order to meet FDA requirements.				
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